InstantScreen^{TM} Rapid HIV-1/2 assay

(Rapid Blood/ Serum/ Plasma-Test for Antibodies to Human Immunodeficiency Virus Type 1 and Type 2)

- For in vitro use only -

Summary

The *InstantScreen*^{TM} Rapid HIV-1/2 assay tests human blood (20 µl), serum or plasma (10 µl) for antibodies to the Human Immunodeficiency Viruses Type 1 and Type 2 (HIV-1 and HIV-2 respectively) in less than 1 minute. The assay is packaged as a kit containing 20 Test Devices, Diluent for blood, serum or plasma, Detector Reagent and Wash Solution as well as lancets and capillaries for collecting blood. *InstantScreen*^{TM} Rapid HIV-1/2 assay can be performed without any additional materials or lab instruments and does not require special skills or medical knowledge. As such, different packaging formats of the test are suitable as a home test or for use in doctor`s offices and hospitals or other agencies. The use of the test by physicians or nurses is recommended because of the need of counselling and confirmatory testing of those patients who have positive test results.

Background Information

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least one of two retroviruses, HIV-1 and HIV-2, collectively called HIV. HIV-1 and HIV-2 are similar in genomic structure, morphology and ability to cause AIDS. HIV is transmitted mainly by sexual contact, needle sharing of i.v. drug users, exposure of open wounds to blood or blood products, or from an infected mother to her foetus. HIV has been isolated from patients with AIDS, AIDS-related complex (ARC), and from persons at high risk of contracting AIDS. Antibodies specific for HIV are prevalent in sera from HIV-infected persons, as well as in people with AIDS or ARC.

Among other factors, the specificity and sensitivity of HIV-antibody tests depends upon: a) the selection of HIV antigens used for antibody detection,

b) the classes of antibodies recognized by the detection reagent,

c) the complexity of the protocol used to perform the test.

The Antigens used in *InstantScreen*^{TM} Rapid HIV-1/2 assay

The ***InstantScreen***^{TM} Rapid HIV-1/2 assay utilizes a combination of a **proprietary "epitope-combi-antigens",** which comprise all major antigenic and conserved epitopes of HIV-1 and HIV-2. The use of these novel „designer proteins" overcomes sensitivity and specificity problems associated with tests based on viral lysates, peptides or unmodified viral proteins. The design of „epitope-combi-antigens" was based upon searches in sequence data banks for both immunogenic epitopes of the humoral immune response and sequences, which are conserved among different strains of HIV.

The construction of these novel proteins was performed by molecular biology techniques (genetic engineering), which resulted in an increased number of highly antigenic epitopes and the elimination of sites, which promote unspecific antibody binding. In addition, specific biochemical procedures are used to unfold the antigen proteins completely. This results in a very high sensitivity and specificity of antibody binding.

Detection System

The ***InstantScreen***^{TM} Rapid HIV-1/2 assay uses a novel detection system to detect antibodies to HIV-1 and HIV-2. This detection system is called **OCA-particles („optimal catch avidity particles")** and shows high sensitivity. Most important is the temperature stability. This allows transportation and storage of the test without cooling into any area of the world. OCA-

particles will detect antibodies during the course of HIV-infection, but also in samples during seroconversion (2-8 weeks after infection).
The ***InstantScreen***™ Rapid HIV-1/2 assay detection fluid and other test components are stable at 45 °C for more than one year or at 60°C for more than 4 weeks. Treatment of the OCA-particle solution at 95 °C for 15 minutes causes only a slight reduction in the assay sensitivity. Freezing and thawing does not impair the function of the reagent. The reagents are ready to use, i.e. they do not need to be reconstituted with water or test solutions.

Intended purpose

InstantScreen™ Rapid HIV-1/2 assay is a screening test, which allows the detection of anti-HIV-antibodies in less than 1 minute. The test can be performed without further instruments or technical support. It is not recommended to use the test at blood banks without additional assays, which test for the presence of HIV directly, since freshly infected persons may not yet have developed antibodies against HIV.
It is internationally accepted that a screening assay should be supplemented by a confirmation assay, which is based upon another test procedure. These are ELISA-antibody tests, Western Blots, the Polymerase Chain Reaction (PCR) or p24-ELISAs.

Simple test protocol

The assay consists of only three steps, which can be completed in less than 1 minute:

Step 1: Mixing of the blood, serum or plasma sample with the Diluent (solution 1) and pouring it into the Test Device.
Step 2: Pouring the Detector (solution 2) into the Test Device.
Step 3: Adding the Wash (solution 3).

The Test Device can be easily opened by twisting and the test membrane can be removed and taped to the patients file for permanent result documentation and prevention of mix-ups.
All reagents and materials required to carry out the test are included. Therefore, a medical laboratory or special skills are not needed to perform the test reliably.

Reagents and Materials

The ***InstantScreen***™ Rapid HIV-1/2 assay Test kit for institutional use contains 20 complete tests, which can be used independently from each other for individual assays. Therefore, there is no need to collect samples prior to the performance of a test. Reagents can be stored at ambient temperature. The Detector (solution 2) should not be exposed to sunlight for more than one hour. It is therefore recommended to close the container after removal of an ***InstantScreen***™ Rapid HIV-1/2 assay set.

Contents	Quantity per box
1. ***InstantScreen***™ Test Devices	20
2. Lancets	20
3. Heparinized 20 µl blood collection capillaries	25
4. Mixing bottles	20
5. Diluent vials (solution 1)	20
6. Detector vials (solution 2)	20
7. Wash vials (solution 3)	20
8. Package Insert	1
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Types of specimen, which can be used for reliable test results

- Fresh blood, obtained according to operating instructions (see below),
- Fresh blood samples with anti-coagulants, i.e. EDTA, citrate or heparin,
- Serum samples stored at 2-4°C under sterile conditions for up to 2 weeks,
- Serum samples stored at –20 C for up to 6 months,
- Plasma samples stored at 2-4°C under sterile conditions for up to 2 weeks,
- Plasma samples stored at -20°C for up to 6 months.

Bacterial contamination of specimens should be avoided, since it may lead to false-positive test results. If degraded specimens are used, the control dot on the left side of the test membrane will not appear. Therefore, the appearance of this control is essential for the validity of the test.

Operating instructions

1. Open the Alu-pack and remove the Test Device.
2. Take a 20 µl-capillary from the capillary container.
3. Take the lancet, puncture the side of the fingertip, fill the capillary with blood completely and transfer it into the mixing bottle.
4. Open the Diluent (solution 1) vial and pour the contents into the mixing bottle.
5. Shake vigorously and pour the complete mixture immediately into the Test Device.
6. After the solution has been absorbed, shake Detector (solution 2) vigorously and pour completely into the Test Device.
7. After the solution has been absorbed, pour all of the Wash (solution 3) into the Test Device.
8. The membrane shows the test result, which is explained on the Test Device.

Interpretation of Results

a) **Negative**: One blue dot appears on the left side of the membrane. This is the control dot, which shows that the test has been performed correctly and that a sufficient amount of antibodies was present in the specimen. A sample giving this pattern is considered negative.
b) **Positive**: Two blue dots indicate that the specimen contains anti-HIV-1 and/or anti-HIV-2 antibodies. A sample giving this pattern is considered positive. Depending upon the volume of the sample, the antibody titer and/or the antibody composition (which varies considerably from patient to patient), the HIV-dot may be more or less intense in colour compared to the control dot.
c) **Invalid**: If the control dot does not appear on the membrane, the test was performed incorrectly or there is a problem with the sample (e.g. antibody degradation). A sample giving this pattern is considered invalid and the test must be repeated.

Limitations of the Test

The ***InstantScreen***^{TM} Rapid HIV-1/2 assay detects antibodies to HIV, which are indicative for an infection with HIV. However, within the first weeks after infection antibodies against HIV may have not yet been produced by the immune system. The duration of this period depends upon a variety of circumstances and may last between 2-8 weeks. However, the incidence of HIV-antibody-negative patients, which are HIV-infected, has been estimated to be 1 in 200.000 in the developed countries. Therefore, the likelihood of missing a HIV-infection using an antibody test is low.
Since a variety of factors may cause non-specific reactions (which, however, have not been found among the patients tested with ***InstantScreen***^{TM} Rapid HIV-1/2 assay so far), samples found to be positive using the ***InstantScreen***^{TM} Rapid HIV-1/2 assay should be retested using a confirmatory test for HIV such as ELISA tests and/or Western Blots and/or PCR. Reactivity in either of these tests confirms an infection with HIV. A person who has antibodies to HIV is presumed to be infected with the virus and appropriate counselling and medical evaluation should be offered. Such an evaluation should be considered an important part of HIV antibody testing and should include test result confirmation on a freshly drawn sample.

Performance Characteristics: Specificity and Sensitivity

The sensitivity of a test is the ability of a test to detect truly infected people, whereas the specificity of a test is the ability of a test to identify all non-infected individuals. Thus, a sensitive test will not produce false-negatives and a specific test will not produce false-positives. There is no single standard for detecting the sensitivity or specificity of an antibody test for HIV-1 or HIV-2 in human sera or plasma. However, the generally accepted method is to express the sensitivity and specificity by comparison with supplemental assays such as ELISA and Western Blots.
The sensitivity of an assay is also reflected in the examination of seroconversion panels, i.e. collections of serum from patients, where the time of HIV-infection was known. Such seroconversion panels allow the evaluation of an assay with regard to the detection of HIV-antibodies being produced in the first stage of infection. These early antibodies consist mainly of the IgM class, while the IgG class dominates at later stages of infection.

Examples of recent studies comparing ***InstantScreen***^{TM} Rapid HIV-1/2 assay which an ELISA or Western Blot are given subsequently:

Samples	***InstantScreen***^{TM} **Rapid HIV-1/2 assay**	**ELISA**
Total number of samples: 1.445	Number of patients	
	(+/-)	(+/-)
HIV-pos. blood	315/0	315/0
HIV-neg. blood	0/312	0/312
HIV-pos. serum	310/0	310/0
HIV-neg. serum	0/299	0/299

Problem-sera:		
Hepatitis B, HIV-pos.	3/0	3/0
Hepatitis B, HIV-neg.	0/83	0/83
Hepatitis C, HIV-pos.	7/0	7/0
Hepatitis C, HIV-neg.	0/64	0/64
CMV, HIV-neg.	0/41	0/41
Pregnancy, HIV-neg.	0/21	0/21

Based upon this study, the test has a sensitivity of 100% and a specificity of 100% even when patients are tested with other infectious diseases, which can cause up to 50% false positive reactions with some laboratory assays.

Results of an on-site field study in Africa in a rural population with high incidence of Malaria, Hepatits B and Tuberculosis are shown below:

Samples	***InstantScreen*™ Rapid HIV-1/2 assay**	**Western Blot**
Total number of samples: 969	Number of patients	
	(+/-)	(+/-)
HIV-pos. blood	19/0	19/0
HIV-neg. blood	0/950	n.a.

Sensitivity studies with ***InstantScreen*™** Rapid HIV-1/2 assay had shown that:
- strongly HIV-positive sera can be detected even if a very small amount (25–50 nl) of serum is used,
- weak HIV-positive sera can be detected by using 1 µl serum.
- Typically, much higher serum amounts (5-10 µl) are used for convenience and safety.

Warnings and Precautions

1. Do not smoke, eat or drink in areas where blood, serum or plasma specimens are being handled.
2. Laboratory coats and latex gloves are recommended while handling any blood, serum or plasma other than your own.
3. Avoid contact of samples with your skin and eyes, in particular with open wounds. Such contact may cause you to become infected. Just a drop of blood from the specimen sample could be sufficient to cause HIV-infection.
4. Blood, serum or plasma samples may contain other transmittable diseases. Therefore, these samples need to be treated as potentially infectious. These agents may be more infectious than HIV, and therefore easily transmittable.
5. Once a pouched ***InstantScreen*™** Rapid HIV-1/2 assay Test Device is opened, it must be used within a couple of hours.
6. Make always sure that all plastic vials (solution 1-3) of the assay are opened immediately prior to use, to prevent any possibility of unauthorized interference with the test performance.
7. Blood collected with the capillary contained in the test must be used within 2 minutes to prevent coagulation within the capillary.
8. HIV will be largely inactivated after mixing with solution 1. However, the diluent mixture should be considered as potentially infectious. The Test Device (after performing the test) is self-contained and does not pose a risk with respect to HIV-infection. However, since other infectious agents may be contained in the sample of the patient, it is recommended to dispose of the spent test accordingly.
9. Do not mix reagents from different lots and do not use kit components, which have passed the expiration date.
10. The assay cannot be used for urine or saliva. Such body fluids can be tested for anti-HIV-antibodies using newly developed rapid assays which GAIFAR will introduce into the market.
11. Prevent prolonged (one hour) exposure of Detector (solution 2) to sunlight, since this may impair the sensitivity of the test.

Areas of administration

- Home test,
- Doctor´s office,
- Emergency rooms, ambulances and life saving services,
- Clinics and clinical laboratories,
- Government agencies and non-governmental health organisations,
- Dentists.

Storage conditions

InstantScreen^{TM} Rapid HIV-1/2 assay can be stored at all ambient temperatures ranging from –20°C to +45°C. Prolonged exposure to sunlight, especially of the Detector (solution 2) must be avoided. Therefore, the complete test kit should be stored in the package. Repeated freezing and thawing, storage at 60°C for at least 4 weeks or at +45°C for at least 18 months as well as at high relative humidity will not influence the test performance, provided the test remains in the original package.
If the package has been damaged following destruction of the package box or if the pouch of the Test Device has been perforated for more than 2 hours, the test sensitivity could be affected.

Shelf life

InstantScreen^{TM} Rapid HIV-1/2 assay is stable at ambient temperature for a period of at least 18 months.

Regulatory information

InstantScreen^{TM} Rapid HIV-1/2 assay has been manufactured based upon the production approval according to the German Drug Law. GMP-Certificate as well as WHO-Certificate for export has been obtained accordingly.

Helpful Handling Recommendations (see also User´s Guide)

How to draw blood ?
Puncture the finger at the side of the fingertip with the lancet enclosed in the test kit. Then keep the whole arm straight down to let a blood drop accumulate, before you let the blood be sucked (through capillary force) into the heparinized capillary, which is also provided.

How to get the capillary out of the glass container ?
Keep the vial upside down and shake slightly until a capillary drops into your hand.

How to get the blood out of the capillary ?
Add the capillary with blood into the mixing bottle and add Diluent (solution 1). Then screw the cap tightly and shake vigorously. The capillary will then release the blood into the Diluent. It is not necessary to wait for the complete lysis of all erythrocytes (clear pink solution) prior to the transfer into the Test Device.

Do all solutions need to be absorbed completely prior to the addition of the next one ?
Yes, since mixing will impair the sensitivity of the assay. Therefore, always wait the 5-10 seconds, which are required for one solution to be completely absorbed into the Test Device before pouring the next solution in.

How to confirm a positive reaction ?
All positive reactions should be confirmed (as it is always done in a clinical laboratory) by an independent confirmatory test.

What is the meaning of the appearance of the control dot ?
The left blue dot is the control dot, which indicates that:
a) blood, serum or plasma has been added in sufficient amount and quality,
b) the test components are functioning properly, i.e. they were not altered by third parties after the purchase of the original test,
c) the test is not a fake copy, but the original.

Should one use a Test Device, which was not obtained from an intact pouch ?
No, the pouches are of the highest quality to ensure that no degradation of the test occurs even at very moist or hot environmental conditions. Prolonged exposure of the open Test Device (many hours or days) may cause changes resulting in less reliable results.

Which component should be always kept in the storage box ?
The blue Detector (solution 2). This solution can loose reactivity, if exposed for hours to direct strong sunlight. Light can cause chemical alterations of some of the ingredients. Therefore, it is recommended to take only as many Detector vials out

of the box as are used within the next couple of hours. Close the box properly after usage.

Should the Test Device be marked with the patient name or code number ?
Yes, the Test Device has the front area specifically made in a way that the name or code number of a patient can be written on the Device along with the date. This will prevent the samples from being mixed up.

Is the test result permanent ?
Yes, the test result is permanent and can be used for a permanent and tampering-proof documentation of the test result in the file of a patient. For this purpose, it is possible to remove the membrane from the Test Device, fix it to the document for drying and finally cover with adhesive plastic film.
Even if the test membrane remains in the Test Device, the results remain stable over days and (depending upon storage conditions) up to weeks. If the developed tests are kept in a closed box, they remain moist. This keeps the intensity of the colour reaction far better than if the tests were allowed to dry out.

If a sediment is visible in the Detector solution – what should be done ?
Shake vigorously to resuspend all colour particles before using it. Sedimentation is normal and occurs within 1-2 days of storage. Therefore, the Detector (solution 2) must be shaken vigorously immediately prior to use.

What are the causes likely for a slight blue background colour after completion of the reaction?
Blue background colour may occur after an extended incubation of lysed erythrocytes within the mixing vial. This background will never exceed a level, which interferes with the quality of the test result. The test works equally well with completely lysed erythrocytes (clear pink solution in the mixing bottle) or intact erythrocytes (up to 1 min. after mixing, turbid pink solution). In the latter case, the membrane can turn red after application of the sample, but clears completely after addition of solution 2 and 3.

Is the amount of fluid in one plastic vial sufficient to perform more than one test ?
No, always use the full content of the plastic vials, which contain solutions 1-3. Never split them for different tests, since the content is specifically adapted for the use in <u>one test only</u>.

Is Wash (solution 3) essential ?
No, not essential, but it takes only 10 more seconds and has significant advantages. In the case of a normal strong reaction, the use of solution 3 can be omitted if serum or plasma is tested. However, using the Wash (solution 3) will clear the background from a slight blue into a white. This means that weakly positive sera can be better recognized. For testing of blood samples, solution 3 should always be used.

<u>Confirmation Testing</u>

GAIFAR GmbH is highly confident with respect to the superior performance of its diagnostics products. However, it needs to be emphasized, that all HIV-screening tests, even if they are 100 % accurate as the ***InstantScreen™*** test has proven to be so far, need to be confirmed by additional tests. The internationally accepted principle of the confirmation testing is the Western-Blot, which tests for the reaction of patient sample with HIV-proteins other than those used, in the screening test. GAIFAR offers the worldwide first rapid (3-5 min.) Western-Blot confirmation tests, which can be performed in the field without additional materials or skills and requires only 2 µl of blood. For the first time, reliable HIV-testing in field is now possible, since no samples need to be shipped to laboratories for confirmation testing.

<u>Counselling</u>

All patients tested HIV-positive need counselling and therapy as well as social and psychological support. Depending upon the circumstances in the country, the social and economic conditions, such counselling will differ in focus and content. Due to the high costs of modern therapies, counselling in many developing countries will focus upon prevention and the use to low cost therapies. Prevention is the highest priority of the "Knowledge Protects" family of GAIFAR diagnostics, because the rapid spread of HIV-infections in developing countries is due to the fact that up to 90 % of the HIV-infected people are unaware of their HIV-infection and have hardly a chance of ever gaining this information if they have no access to rapid HIV-testing. Therefore, the rapid spread of HIV-infections in developing countries can be only reduced if the number of people who

infect others, unaware of being infected themselves, is dramatically reduced. This requires not only precision of screening and confirmation testing but also complete confidentiality of testing and counselling. Both requirements are met with the

subsequent use of ***InstantScreen***™ (screening test) and *InstantConfirm*™ (confirmation test).

Legal Disclaimer

For legal reasons, GAIFAR cannot accept any liability for the results of ***InstantScreen***™ or other members of the "Knowledge Protects" family of rapid diagnostics. This includes also the implications of such tests. Therefore, all liability is limited to the replacement of the test. By using the product, the user indemnifies and holds GAIFAR GmbH, its agents, personnel and/or its successors harmless against any legal action instituted by or against or on behalf of the user. Specifically, but not exclusively this includes all costs incurred by the user, on behalf of the user or by other parties if such claims are brought against the user by third parties as far as they are connected directly or indirectly with the outcome of any tests of "Knowledge Protects" family.

GAIFAR has designed its test in a way that complete confidentiality of testing and counselling can be achieved and that tempering with the test itself is almost impossible. However, the use of "Knowledge Protects" diagnostics, specifically but not exclusively ***InstantScreen***™ and *InstantConfirm*™, implies that the user indemnifies and holds harmless GAIFAR to actions of third parties with regard to conduct of the tests and handling of its results.

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